Remarks

The Office Action mailed December 31, 2002 has been received and reviewed. Claims 1 and 9-11 having been amended, the pending claims are claims 1-20 and 77-81. Support for amended claims 1 and 9-11 is found on p. 4, lines 17-19 of the specification. Applicants thank the Examiner for acknowledging that claims 4, 5, and 7 are free of the art. Reconsideration and withdrawal of the rejections are respectfully requested.

The 35 U.S.C. §112, Second Paragraph, Rejection

The Examiner rejected claims 1-20 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Specifically, the Examiner asserted that the recitation "meaningful difference" in claims 1 and 9-11 was vague and indefinite, that the term is subjective because its metes and bounds are determined by the individual practitioner.

This rejection is respectfully traversed. Amended claims 1 and 9-11 recite to a "mathematically significant difference between the two groups of animals in the survival rates, levels of microbes, or levels of infection present." Applicants submit that the metes and bounds of a "mathematically significant difference between the two groups of animals in the survival rates, levels of microbes, or levels of infection present" in the methods of claims 1-20 are well understood to one of skill in the art. As explained in the specification, on page 16, lines 18-20, such a difference can be "quantified with well known statistical tests" and can be "determined by one [of] ordinary skill in the art of evaluating microbial infections." Examples of the determinations of such a "mathematically significant difference" by those of ordinary skill in the art of evaluating microbial infections are demonstrated, for example, in Brown et al., "Signature-tagged and directed mutagenesis identify PABA synthetase as essential for Aspergillus fumigatus pathogenicity," *Mol Microbiol.* 2000 *36*(6):1371-1380 (see "Statistical Analysis," p. 1379); Hensel et al., "Simultaneous identification of bacterial virulence genes by negative selection," *Science* 1995 21;269(5222):400-403 (see Table 1, discussion of the

determination of LD_{50}); and Shea et al., "Identification of a virulence locus encoding a second type III secretion system in Salmonella typhimurium," *Proc Natl Acad Sci U S A.* 1996 19;93(6):2593-2597 (see p. 2596, col. 1).

Applicants submit that the recitation "mathematically significant difference between the two groups of animals in the survival rates, levels of microbes, or levels of infection present" has a clear meaning to those of ordinary skill in art. Withdrawal of the rejection of claims 1-20 under 35 U.S.C. §112, second paragraph, is respectfully requested.

The 35 U.S.C. §103 Rejection

The Examiner rejected claims 1-3, 6, 8-20, and 77-80 under 35 U.S.C. §103(a) as being unpatentable over Bostian et al. (WO 96/40979) in view of Setterstrom et al. (U.S. Patent No. 6,309,669 B1) and Burnham et al. (U.S. Patent No. 5,891,670) or Nesin et al. ("Cloning and Nucleotide Sequence of a Chromosomally Encoded Tetracycline Resistance Determinant, *tetA*(M), from a Pathogenic, Methicillin-Resistant Strain of *Staphylococcus aureus*," *Antimicrobial Agents and Chemotherapy;* 1990, *34*:2273-2276). This rejection is respectfully traversed.

Applicants submit that the Examiner's statement of the rejection is unclear. In rejecting claims 1-3, 6, 8-20, and 77-80 under 35 U.S.C. §103(a), the Examiner asserted that it would have been obvious to one of ordinary skill in the art to combine the teachings of Bostian et al. with those of Setterstrom et al. and, also asserted that it would have been obvious to one of ordinary skill in the art to combine the teachings of Bostian et al. with either of Burnham et al. or Nesin et al. (see p. 9, Office Action mailed December 31, 2002). Is it the Examiner's position that the claimed invention is unpatentable over Bostian et al. in view of any one of Setterstrom et al., Burnham et al. or Nesin et al.? Or, is it the Examiner's position that the claimed invention is unpatentable over Bostian et al. in view of Setterstrom et al. and further in view of either Burnham et al. or Nesin et al.? Clarification is requested. Applicants have assumed the latter in making the following comments.

The burden is on the Examiner to establish the prima facie case of the obviousness of the claimed invention, and it is respectfully argued that the Examiner has fallen short of meeting this burden. In particular, the Examiner has failed to establish at least two of the basic criteria of a prima facie case of obviousness. One, the Examiner has failed to establish that there is a suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to combine the teachings of the cited references. Two, the Examiner has failed to establish that the prior art references, when combined, teach or suggest all the claim limitations. See MPEP § 2143.

The Examiner asserted that it would have been prima facia obvious to modify the methods of Bostian et al. for the characterization of potential antimicrobial gene targets to include the use of control animals, as taught in Setterstrom et al. On page 9 of the Office Action mailed December 31, 2002, the Examiner asserted that it would have been obvious to modify the methods taught by Bostian et al. to include the control animals "because Bostian et al. teach that it is within the skill of the art to utilize a tetracycline-responsive system to control the level of activity of a gene target in a microbe during the infection process and because Setterstrom et al. teach it is within the skill of the art to utilize a control animal to provide a clear contrast between treatment and nontreatment." The Examiner asserted that "[o]ne would be motivated to do so in order to receive the expected benefit, as exemplified by Setterstrom et al.," of being able to compare the level of infection between treated and untreated animals.

Applicants respectfully disagree. The Examiner asserted that the motivation to combine the cited teachings would be "in order to receive the expected benefit" of such combination. Applicant's submit that Setterstrom et al. teach the use of untreated control animals in one very specific assay, an assay of the effectiveness of microencapsulated antibiotics, such as ampicillin, for the treatment of osteomyelitis (a bone infection) (see Ex. 1-7). Setterstrom et al. provide no teachings or motivation to extend the use of such untreated controls to other assay systems. Bostian et al. provide no teaching or motivation of a need to improve on its methods for the characterization of potential antimicrobial gene targets. Applicants submit

that the requisite motivation to combine the teachings of the cited references can not be found in either in the references themselves or in the knowledge generally available to one of ordinary skill in the art. With this rejection the Examiner has used impermissible hindsight to reconstruct the claimed invention.

Further, Applicants submit that even if one of skill in the art were to combine the teachings of Bostian et al. with the teachings of Setterstrom et al., which they would not be motivated to do, they would not obtain the invention of claims 1-3, 6, 8-20, and 77-80. The combination of Bostian et al. with Setterstrom et al. would yield two separate groups of animals. The first group of animals would be those animals treated with an antibiotic during the entire course of the experiment. The second group of animals would be the "control" animals, those animals to which an antibiotic was never administered. This is not the same as the claimed invention.

Claims 1-3, 6, 8-20, and 79-80 are drawn to a process comprising: "infecting a plurality of mammals with a microbe that has been genetically altered" (see claim 1, line 4); "exposing the plurality of mammals to tetracycline" (see claim 1, line 13); and "once an infection with the genetically altered microbe is established, removing the tetracycline exposure of a portion of the plurality of mammals, such that a first group of the plurality of mammals is exposed to tetracycline and a second group of the plurality of mammals is not exposed to tetracycline" (see claim 1, lines 14-17). Thus, the two groups of the claimed invention are a first group that is exposed to tetracycline for the entire course of the process, and a second group that is initially exposed to tetracycline, followed by removal of the tetracycline treatment. The two groups of the claimed invention are very different from the two groups obtained by the combined teachings of Bostian et al. with Setterstrom et al.

Claims 77 and 78 are drawn to a "process to regulate expression of a gene product by a microbe in a mammalian host . . . comprising[:] infecting a mammalian host with a microbe that has been genetically altered such that the amount of said gene product produced by said genetically altered microbe is regulated by a Tetracycline-Controllable Element (TCE)" and

"where said genetically altered microbe also comprises a polynucleotide sequence encoding a tetracycline resistance protein." Neither of Bostian et al. nor Setterstrom et al. teach a genetically altered microbe which comprises a polynucleotide sequence encoding a tetracycline resistance protein. Thus, Applicants submit that the combination of the teachings of Bostian et al. with the teachings of Setterstrom et al. does not result in the claimed process of claims 77 and 78, a process comprising an altered microbe comprising both a TCE and a polynucleotide sequence encoding a tetracycline resistance protein.

For the reasons discussed above, Applicants respectfully submit one of ordinary skill in the art would not have been motivated to combine the teachings of Bostian et al. in view of Setterstrom et al., and even if combined, the teachings of Bostian et al. and Setterstrom et al. would not teach or suggest the claimed invention. Further, the teachings of either of Burnham et al. or Nesin et al. do not correct for these deficiencies.

The Examiner asserted that it would have been obvious to one of ordinary skill in the art to modify the genetically altered microbe taught by Bostian et al. to include a gene encoding tetracycline resistance, as taught by either of Burnham et al. or Nesin et al. (see p. 9 of the Office Action mailed December 31, 2002). The Examiner asserted that one would have been motivated to do so "in order to avoid complications in interpreting the experimental data upon addition/withdrawal of the tetracycline 'switching' compound" (see p. 10 of the Office Action mailed December 31, 2002). Applicants respectfully disagree.

First, Applicants note that it is unclear from the record whether the Examiner has rejected the claims as obvious over the teachings of Bostian et al. alone in view of either of Burnham et al. or Nesin et al, or whether the Examiner has rejected the claims as obvious over the teachings of Bostian et al. in view of Setterstrom, further in view of either of Burnham et al. or Nesin et al. Clarification is requested.

Regardless, Applicants respectfully submit that the Examiner has failed to establish that there is a suggestion or motivation, either in the documents themselves or in the knowledge generally available to one of ordinary skill in the art, to combine the teachings of

Burnham et al. or Nesin et al. with the teachings of either Bostian et al. or Bostian et al. in view of Setterstrom et al. Bostian et al. teach that the tet repressor/tet operator can be used as a switch (p. 54, lines 15-17) in methods for evaluating specific microbial genes and teaches that tetracycline is an example of a switching compound that can be used in these methods (p. 17, lines 11-13). However, when discussing the use of tetracycline as a switching compound, Bostian et al. teach that "[i]n certain bacteria, exposure to a low level (sub-growth inhibitory level) of tetracycline induces a much-elevated level of expression of a gene from the resistance-related promoter" (p. 17, lines 13-16). Thus, as Bostian et al. teach the use of tetracycline as a switching compound at low level, sub-growth inhibitory concentrations, Bostian et al. provide no teachings of a need (motivation) to add a polynucleotide sequence encoding a tetracycline resistance protein into the microbe. Setterstrom et al. contains no teachings of tetracycline resistance or antibiotic resistence in general. Thus, Applicants submit that the requisite motivation to combine the teachings of Burnham et al. or Nesin et al. with Bostian et al. (or with Bostian et al. in view of Setterstrom et al.) can not be found in either the documents themselves or in the knowledge generally available to one of ordinary skill in the art.

For the reasons discussed above, Applicants respectfully submit that claims 1-3, 6, 8-20, and 77-80 are not unpatentable over Bostian et al. in view of Setterstrom et al. and Burnham et al. or Nesin et al. Withdrawal of this rejection under 35 U.S.C. §103(a) is respectfully requested.

Summary

It is respectfully submitted that the pending claims 1-20 and 77-81 are in condition for allowance and notification to that effect is respectfully requested. The Examiner is invited to contact Applicants' Representatives, at the below-listed telephone number, if it is believed that prosecution of this application may be assisted thereby.

Respectfully submitted for
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By: _____
Name: SAM HER

APPENDIX A - SPECIFICATION/CLAIM AMENDMENTS INCLUDING NOTATIONS TO INDICATE CHANGES MADE
Serial No.: 09/600,392
Confirmation No.: 4850
Docket No.: 6137.P US

Amendments to the following are indicated by underlining what has been added and bracketing what has been deleted. Additionally, all amendments have been shaded.

In the Claims

1. [AMENDED] A process for the identification of a microbial gene encoding a gene product that is important to a microbe's ability to infect or sustain an infection in a mammal, which process comprises:

infecting a plurality of mammals with a microbe that has been genetically altered such that the amount of said gene product produced by said genetically altered microbe is regulated by a Tetracycline-Controllable Element (TCE);

where said TCE is a gene regulatory system that controls the expression of the target gene product through its ability to modulate the function of said gene in response to said microbe's exposure to tetracycline, and where said TCE is comprised of a tetracycline-controllable transcription promoter polynucleotide sequence;

where said genetically altered microbe also comprises a polynucleotide sequence encoding a tetracycline resistance protein;

exposing the plurality of mammals to tetracycline;

once an infection with the genetically altered microbe is established, removing the tetracycline exposure of a portion of the plurality of mammals, such that a first group of the plurality of mammals is exposed to tetracycline and a second group of the plurality of mammals is not exposed to tetracycline; and

comparing the degree of infection, microbe levels, or survival rates of the mammals in the first group and the second group wherein a **[meaningful] mathematically significant** difference between the two groups of animals **in the survival rates, levels of microbes, or levels of infection present** identifies the gene product as important to a microbe's ability to infect or sustain an infection in a mammal.

2. The process of claim 1, where said TCE is operably linked to a polynucleotide sequence encoding a reporter gene (RG).

3. The process of claim 1, where said tetracycline-controllable transcription promoter polynucleotide sequence is a prokaryotic transcription promoter.

4. The process of claim 1, where said TCE is operably linked to a polynucleotide sequence encoding a reporter gene (RG) and a target gene (TG).

5. The process of claim 4, where said reporter gene encodes a β-lactamase.

6. The process of claim 1, where said polynucleotide sequence encoding a tetracycline resistance protein is contained on a tetracycline resistance and repressor DNA cassette (TRRDC), said TRRDC comprising a tetracycline repressor gene and a tetracycline resistance gene.

7. The process of claim 6, where said TCE is operably linked to a polynucleotide sequence encoding a reporter gene (RG) and a target gene (TG) and where the TCE, the TRRDC, the RG, and the TG are all on the same DNA cassette, referred to as a Regulatory DNA Cassette (RDC).

8. The process of claim 6, where said TRRDC promoter is operably linked to the TCE, the tetracycline repressor gene comprises the structural gene *tetM*, and the tetracycline resistance gene comprises the structural gene *tetR*.

9. [AMENDED] The process of claim 1, where said **[meaningful]** **mathematically significant** difference between the two groups of animals is a **[meaningful]** **mathematically significant** difference in the levels of microbes or levels of infection present in the mammals.

10. [AMENDED] The process of claim 1, where said **[meaningful] mathematically significant** difference between the two groups of animals is a **[meaningful] mathematically significant** difference in the survival rates of the groups of animals.

11. [AMENDED] The process of claim 1, where said **[meaningful] mathematically significant** difference between the two groups of animals shows that animals exposed to tetracycline have poorer health, higher rates of infection, lower survival or higher levels of microbes than animals not exposed to tetracycline.

12. The process of claim 6, where said tetracycline resistance gene of said TRRDC comprises sequences from the *Staphylococcus aureus tetM* gene.

13. The process of claim 6, where said tetracycline repressor gene of said TRRDC is obtained from the Tn10 transposon.

14. The process of claim 6, where said TRRDC comprises the sequence of SEQ ID NO:35 or SEQ ID NO:36.

15. The process of claim 1, where said infected mammals are mice.

16. The process of claim 1, where said genetically altered microbe is a Staphylococcus species.

17. The process of claim 16, where said Staphylococcus species is *Staphylococcus aureus*.

18. The process of claim 1, where said microbe is a virus.

19. The process of claim 1, where said microbe is a lower eukaryote.

20. The process of claim 1, where said microbe is a yeast.

77. A process to regulate expression of a gene product by a microbe in a mammalian host with tetracycline or a tetracycline analog, said process comprising:

infecting a mammalian host with a microbe that has been genetically altered such that the amount of said gene product produced by said genetically altered microbe is regulated by a Tetracycline-Controllable Element (TCE);

where said TCE is a gene regulatory system that controls the expression of the target gene product through its ability to modulate the function of said gene in response to said microbe's exposure to tetracycline, and where said TCE is comprised of a tetracycline-controllable transcription promoter polynucleotide sequence;

where said genetically altered microbe also comprises a polynucleotide sequence encoding a tetracycline resistance protein; and

exposing the mammalian host to tetracycline.

78. The process of claim 77, further comprising, once an infection with the genetically altered microbe is established, removing the tetracycline exposure of the mammalian host.

79. The process of claim 1, where said plurality of mammals are exposed to tetracycline while being infected with the genetically altered microbe.

80. The process of claim 1, where said plurality of mammals are exposed to tetracycline by adding tetracycline to the drinking water.

81. The process of claim 2, where said reporter gene encodes a β-lactamase.